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Crossing the Barrier: STRA6 in Epidermal Differentiation

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In this issue, Skazik *et al.* demonstrate that the STRA6 retinol transporter protein regulates the proliferation and differentiation of epidermal keratinocytes. In human organotypic three-dimensional skin and skin reconstitution models, depletion of STRA6 induced hyperproliferation-associated differentiation, resulting in epidermal expansion. This reveals that STRA6 functions as a "gatekeeper" in retinol (vitamin A)-mediated differentiation of human skin.

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The biological role of retinol

Retinol and its metabolite, all-trans retinoic acid (RA), are essential for embryonic development (Mongan and Gudas, 2007). Humans cannot synthesize retinol, which is consequently obtained from the diet in the form of retinol, retinyl esters, or beta-carotene. Mutations in STRA6, a transmembrane retinol transporter, can result in Matthew–Wood syndrome, and impaired STRA6 function is associated with mental retardation and severe pathological phenotypes in the eye, brain, lung, and heart (Chassaing *et al.*, 2013). In addition to these roles in human disease and development, Skazik *et al.* (2014) used human skin reconstitution models to demonstrate that STRA6 regulates retinol homeostasis in human epidermal keratinocytes.

Crossing the membrane

Retinol is obtained from the diet and enters the bloodstream. Circulating retinol is bound by retinol binding protein 4 (RBP4), and STRA6, an RBP4 receptor, facilitates the transfer of retinol across the cell membrane and delivers it to the cellular retinol binding protein 1 (CRBP1). After entering the cell, retinol is converted into RA by oxidizing enzymes (retinol- and retinal-dehydrogenases). This active form of retinol enters the nucleus, where it binds to a subtype of nuclear receptor termed retinoic acid receptors (RARs).

The receptors take over

Inside the cell, RA elicits a number of responses, including transcriptional activation of numerous genes, ultimately leading to cellular differentiation and growth inhibition. This is achieved by the binding of RA to heterodimers consisting of RARs (RARα, RARβ, and RARγ) and retinoid X receptors (RXRs). The DNA-bound RARs then induce transcription of target genes, including STRA6 and RARβ$_2$, which results in a positive feedback. The transcriptional activation of STRA6 and RARβ$_2$ in embryonic stem cells requires RARγ (Kashyap *et al.*, 2013), which is also the predominant RAR in mouse keratinocytes, where it acts as a tumor suppressor (Chen *et al.*, 2004).

Skin squamous cell carcinoma

RA is currently being used in cancer therapies in which a successful outcome depends on the cancer cells' ability to respond to RA. The resistance of certain cancers to RA is recapitulated in the human prostate cancer cell line PC-3. Normal prostate epithelial cells increase STRA6 transcript levels in response to retinol/RA, leading eventually to growth arrest, whereas PC-3 tumor cells continue to proliferate (Cai and Gudas, 2009). The RA-induced growth inhibition of mouse keratinocytes requires RARγ, and ablation of RARγ predisposes keratinocytes to squamous cell carcinoma (Chen *et al.*, 2004). It would be interesting to know whether this tumor suppressor function of RARγ is related to RARγ's ability to induce transcription of STRA6 in certain cell types.

STRA6 facilitates retinol/RA responsiveness in human keratinocytes

Skazik *et al.* (2014) started out by determining STRA6 expression and retinoid responsiveness in normal human epidermal keratinocyte (NHEK) cells. They next used NHEK cells isolated from three independent donors to demonstrate that only retinol and RA (all-trans, 9-cis, and 11-cis) consistently induced STRA6 expression. Importantly, the STRA6 induction was diminished in the presence of an RA antagonist (LE540). This argues that STRA6 is induced through RAR/RXR heterodimers, either directly or via other RA target genes. Skazik *et al.* (2014) found that STRA6 depletion does not impair cellular uptake of retinol in adherent cultures of HaCaT cells. In contrast, when HaCaT cells were used in human organotypic three-dimensional (3D) skin models, the STRA6-depleted conditions resulted in a significantly thicker epidermis. This was associated with an expansion of the Krt16, Krt6, and Krt10 expression domains. These epidermal markers, which are usually restricted to the upper granular epidermal layer of normal skin, were found throughout the stratum spinosum layer of STRA6-depleted skin. The expanded expression was associated with increased proliferation of the basal epidermal layer, evident by elevated Ki67 expression. Importantly, the elevated levels of Krt16 and, to some extent, the epidermal thickening, were reduced in retinol-supplemented conditions. Skazik *et al.* (2014) further used a human skin reconstitution model to demonstrate that STRA6 depletion *in vivo* is associated with a highly disorganized epidermis and massive epithelial thickening. In summary, Skazik *et al.* (2014) demonstrate that in human organotypic 3D skin and skin reconstitution models, depletion of

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Clinical Implications

- Depletion of STRA6, a transmembrane retinol transporter, results in epidermal thickening.
- Skin regeneration and wound healing could potentially be improved by targeting STRA6.
- Pharmacologically enhancing STRA6 activity may improve cancer differentiation therapies.

STRA6 is associated with elevated levels of proliferation markers. The increased proliferation could allow expansion of the pool of progenitor cells and lead to the observed epidermal thickening.

STRA6 in tissue regeneration

In brief, processes such as digit regeneration in newts and wound healing in mammals are preceded by the generation and thickening of an epithelial cover. Next, the underlying progenitor cells proliferate, thereby providing a reservoir of cells for subsequent tissue repair. This reservoir, commonly referred to as a blastema, is strictly dependent on retinol at all stages of regeneration, including cell cycle initiation and differentiation. The dependence on retinol points to a potential involvement of STRA6. In keratinocytes, STRA6 depletion results in hyperproliferation, which may mirror the initial process of wound healing. Skazik *et al.* (2014) observed an increased rate of closure of scratch wounds of STRA6-depleted HaCaT cultures as compared with controls. Importantly, the levels of Krt16, a marker of epidermal injury and hyperproliferation, were elevated in STRA6-depleted cells. Pharmacological inhibition of STRA6 could therefore be one approach to improve skin regeneration and wound healing.

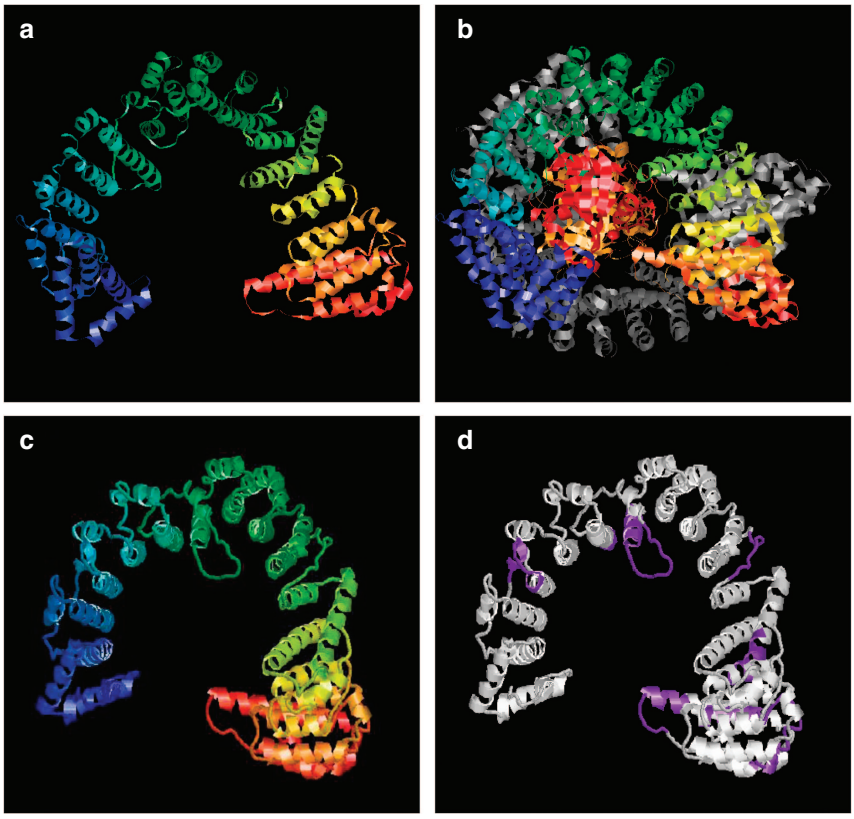

Figure 1. Computational rendering: STRA6 adapts an Importin-like structure. (**a**) STRA6 three-dimensional (3D) structure as predicted by unsupervised computational modeling. (**b**) Importin heterodimer (rainbow and gray) occupied by Ran proteins (red and orange). (**c**) STRA6 3D structure for comparison with (**d**) showing evolutionary divergent regions in purple. This structural prediction could aid in refining the current 9-transmembrane-helical model, and may even suggest specific targets for pharmacological modulation of STRA6 activity. Rainbow-colored scheme applied from the amino- (blue) to the carboxy-terminus (red). Importin: Protein Data Bank 3EA5, predictions: http://zhanglab.ccmb.med.umich.edu/I-TASSER/, graphics: http://www.rasmol.org/.

Modeling of the STRA6 protein

Successful pharmacological approaches rely on detailed insights into the structure and the molecular mechanisms of action of target proteins. The crystal structure of STRA6 has yet to be determined, but unguided 3D prediction suggests an Importin-like structure (Figure 1). This potentially refines the 9-transmembrane-helix model, which was proposed based on mutagenesis and accessibility studies (Zhong *et al.*, 2013). The most recent study evaluated the effects of Cys-biotin insertions to identify two groups of key residues, each of which is predicted to cluster together on one site of an alpha-helical structure (Zhong *et al.*, 2013). A similar arrangement is predicted by the Importin-like model, but the helical structures are shorter, and each helix contains the entire group of important residues (G290-L303 and S385-R394, respectively). In addition, two functionally important distal residues (V319, V320) were identified (Zhong *et al.*, 2013). These are predicted by the Importin-like model to have structurally stabilizing positions, which would explain their importance for STRA6 function. The Importin-like model further predicts that a number of nonessential residues (V365-H384) reside in an outward-facing region of STRA6. Finally, all evolutionarily divergent regions are located in loop regions of the Importin-like STRA6 model (Figure 1). Although these points suggest an Importin-like model, the structure and the mechanism of action of STRA6 will remain unclear until a crystal structure is obtained.

Future directions

Over the past 2 years, the number of STRA6-related papers on PubMed Central has more than doubled. This

increase reflects a growing interest in the cellular uptake of retinol, an area we are just beginning to understand. The lack of good *in vitro* models and the complexity of *in vivo* systems make it a challenging task to unravel the mechanisms of retinol uptake. Our understanding of STRA6 function was increased significantly by recent advances in cellular (Skazik *et al.*, 2014; Zhong *et al.*, 2013) and biological models (Ruiz *et al.*, 2012; Berry *et al.*, 2013). The generation of STRA6-null mice provides a cornerstone for additional biological studies. Three independent groups generated STRA6-null mice, and reported marked ocular defects in them (Ruiz *et al.*, 2012; Berry *et al.*, 2013). Thus, STRA6 is essential for retinol homeostasis in the eye. Importantly, similar ocular defects were observed in humans harboring mutations in STRA6 (Chassaing *et al.*, 2013). The reports on the STRA6-null mice argue that STRA6 is not the only pathway for retinol uptake (Ruiz *et al.*, 2012; Berry *et al.*, 2013). This sets off a search for additional transmembrane retinol transporters. In this respect, it is interesting to note that the Importin-like model of STRA6 predicts heterodimeric complexes (Figure 1), possibly with an unidentified transporter. One candidate for this protein is the recently identified RBP4 receptor-2 (RBPR2), a protein with 40% similarity to STRA6 (Alapatt *et al.*, 2013). Alternatively, RBPR2 may provide a pathway for retinol uptake which functions independently of STRA6 (Berry *et al.*, 2013). Obtaining a crystal structure of STRA6 will be a very important step in revealing its mechanism of action, but this must be complemented with studies identifying STRA6-interacting proteins.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Calcium, Orai1, and Epidermal Proliferation

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Ca^{2+} influx controls essential epidermal functions, including proliferation, differentiation, cell migration, itch, and barrier homeostasis. The Orai1 ion channel allows capacitive Ca^{2+} influx after Ca^{2+} release from the endoplasmic reticulum, and it has now been shown to modulate epidermal atrophy. These findings reveal new interactions among various Ca^{2+} signaling pathways and uncover novel functions for Ca^{2+} signaling via the Orai1 channel.

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Epidermal Ca^{2+} has long been recognized as an essential signal for many epidermal functions. Beginning with early descriptions of the keratinocyte differentiation response, changes in extracellular and intracellular Ca^{2+} have been shown to direct keratinocyte proliferation, differentiation, and barrier homeostasis (reviewed in Mascia *et al.* (2012)). The marked Ca^{2+} gradient present in the epidermis, almost 4-fold higher in the stratum granulosum than in the basal layer, suggests that Ca^{2+} signaling seen in the culture dish is reflected in the *in vivo* responses of the epidermis. This report, ''Reversal of Murine Epidermal Atrophy by Topical Modulation of Calcium Signaling'', by Darbellay *et al.* (2014) reveals that Ca^{2+} flux through the plasma membrane Orai1 channel additionally controls epidermal proliferation and thickness, particularly when the epidermis atrophies in response to aging or chronic corticosteroid topical application. Related recent reports demonstrate further that the Orai1 channel also controls keratinocyte focal adhesion turnover (Vandenberghe *et al.*, 2013) and modulates early aspects of keratinocyte differentiation (Numaga-Tomita and Putney, 2013).

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